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Original Article

Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment

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Aim: Continuous lamivudine treatment is associated with high frequency of drug resistance. We analyzed the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatitis (BTH) in hepatitis B virus (HBV) DNA positive patients receiving lamivudine for > 1 year and correlated it with HBV DNA and alanine aminotransferase (ALT) levels to evaluate if these measurements can provide a practical option for monitoring patients in clinical practice and define early switch from lamivudine therapy.

Methods: Of the 929 patients receiving lamivudine for > 1 year, 359 patients who maintained an ALT level of ≤ 40 IU/L during the course of lamivudine treatment were stratified into two groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years and the other for ≥ 3 years.

Results: The incidence of YMDD motif in patients receiving lamivudine for < 3 years was 27% in patients with ALT ≤ 20 IU/L, 58% with ALT ≤ 30 IU/L, and 63% with ALT ≤ 40 IU/L, ($P = 0.002$). The corresponding incidence of BTH was 2%, 7%, and 48% ($P < 0.001$). The incidence of YMDD motif and BTH in these patients was 7% and 2% with HBV DNA < 2.6 (log copies/mL) and ALT ≤ 20 IU/L, while with ALT at 21–30, the YMDD motif mutant was 16% and BTH was 0%.

Conclusion: Correlation of ALT and HBV DNA levels with YMDD motif mutant and BTH indicates that these measurements can be used in clinical practice for deciding early switch from lamivudine to other suitable antiviral therapies.

Key words: alanine transaminase, breakthrough hepatitis, hepatitis B virus, lamivudine, mutation, viral DNA

INTRODUCTION

LAMIVUDINE HAS GAINED increasing popularity since its approval in 1998 for the treatment of chronic hepatitis B virus (CHBV).[1–4] Lamivudine blocks HBV replication, reduces HBV DNA levels, normalizes alanine aminotransferase (ALT) levels, thereby resulting in histological improvement of the liver.[5] It is a reverse transcriptase inhibitor that acts by competing with the natural polymerase substrate deoxycytidine triphosphate (dCTP) and thus inhibits the elongation of HBV DNA minus strand. It incorporates into the nascent DNA strand and thereby acts as a chain terminator. Although lamivudine is very effective in inhibiting viral replication, the incidence of resistance is high, with an estimated 14–32% of patients developing resistance after 1 year of treatment, 38% after 2 years of treatment, and 53–76% after 3 years of treatment.

Resistance to lamivudine, which increases over years is due to development of mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the DNA polymerase/reverse transcriptase, which is the main target of lamivudine.[4,6–9] This amino acid sequence in YMDD motif is predominantly involved in deoxynucleoside triphosphate (dNTP) binding in the catalytic site of the HBV DNA polymerase.

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Table 1 2007 Ministry of Health, Labour and Welfare of Japan guidelines for hepatits B virus (HBV)-positive patients for nucleoside analogue treatment for patients with chronic HBV receiving lamivudine therapy

Lamivudine therapy HBV DNA		< 3 years	≥ 3 years
Keep < 2.6 log copies/mL		Switch to entecavir 0.5 mg/day	Continue lamivudine 100 mg/day
≥ 2.6 log copies/mL	No BTH†	Switch to entecavir 0.5 mg/day	
	With BTH	Adefovir 10mg/day (duo therapy with lamivudine)	Adefovir 10 mg/day (duo therapy with lamivudine)

†After checking for absence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutation. BTH, breakthrough hepatitis.

Long-term lamivudine therapy is associated with amino acid substitutions mainly in the YMDD motif and also in the proximal FLLAQ (phenylalanine, leucine, alanine, glutamine) motif.[10] Common mutation may occur in the YMDD motif where the methionine residue is replaced either by valine (rtM204V) or isoleucine (rtM204I).[11] These amino acid substitutions form the basis of emergence of lamivudine-resistant strains of HBV and when these occur, the clinical condition may worsen, which is usually accompanied by increase in viral load and serum aminotransferase levels. YMDD mutants cause breakthrough hepatitis (BTH) and, therefore, require withdrawal or switch-over from lamivudine treatment. The American Association for the Study of Liver Diseases (AASLD) and the United States Algorithm for Management of Patients with Drug Resistance recommend either switching over to entecavir or adding adefovir in the event of lamivudine resistance.[12] The 2007 Japanese guidelines of the study group (Ministry of Health, Labour and Welfare of Japan)[13] on standardization of treatment for HBV positive patients for nucleoside analogue treatment for patients with CHBV receiving lamivudine therapy are explained below and also summarized in Table 1.

According to the 2007 guidelines for patients on lamivudine therapy, switching over criteria from lamivudine therapy has been changed from BTH to HBeAg status in patients maintaining HBV DNA copies ≥ 2.6 log copies/mL. Patients on lamivudine for < 3 years and maintaining HBV DNA copies ≥ 2.6 log copies/mL can be switched over to entecavir 0.5 mg/day if they are also HBeAg negative, whereas HBeAg-positive patients can be co-administered adefovir 10 mg/day in both the treatment duration groups (> 3 years or < 3 years).

Unfortunately, the cost of measuring HBV resistance to lamivudine by molecular methods is high and is not presently covered by Japanese reimbursement system in clinical practice. Development of HBV resistance to lamivudine is typically indicated by an increase in HBV DNA followed by an increase in serum ALT levels. Increase in HBV DNA represents active viral replication whereas serum ALT levels provide an indirect assessment of the degree of liver injury.[14]

Hence, in this study, we analyzed the correlation of the incidence of YMDD motif mutant and BTH with HBV DNA and serum ALT levels, either separately or together, in HBV DNA-positive patients who are treated with lamivudine for ≥ 1 year and who had maintained an ALT level of ≤ 40 IU/L until the development of BTH during the course of lamivudine treatment.

METHODS

Patients

THIS WAS A retrospective, nonrandomized study that enrolled 929 HBV DNA-positive-patients receiving 100 mg of lamivudine daily and followed up for a period of 1 year or longer between 1995 and 2006. Since long-term treatment with lamivudine was associated with a high frequency of YMDD motif mutant and BTH (BTH can be defined as abnormal variations in serum transaminase level due to YMDD motif mutant), we analyzed patients who had a possibility to switch to other nucleoside analogues. Patients (n = 395) with ALT ≤ 40 IU/L during follow-up (for 48 patients who developed BTH, data was used until 1 month before the patient developed BTH). Patients were not treated with either adefovir or entecavir during follow-up (for patients who used adefovir or entecavir because of BTH development, data was used until the point before the patient started adefovir or entecavir treatment). Patients were negative for anti-hepatitis C virus (HCV) (third-generation enzyme immunoassay; Chiron, Emerville, CA) and negative for HCV RNA with PCR (Amplicor; Roche Diagnostic Systems, Pleasanton, CA), did not have hepatocellular carcinoma, nore other forms of liver injury such as hemochromatosis, Wilson's disease,

primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease.

Informed consent was obtained from each patient included in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Patients were stratified into 2 groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). In addition, we also analyzed patients based on their ALT level (IU/L) grouped into ≤20, 21–30, and 31–40, and HBV DNA (log copies/mL) divided into < 2.6, 2.6–5.0, and ≥ 5.1.

During treatment, patients were followed up each month for liver function and serum markers of HBV infection. The serum sample of the patients were collected and preserved at −80°C. All the collected samples up to this time period were analyzed for HBV DNA in June 2001. From July 2001, the serum samples were collected and analyzed once a month at the clinical treatment facility.

YMDD motif mutants were determined at the baseline and monitored at 6 months and during the study as well as at the development of breakthrough hepatitis. YMDD motif mutants were analyzed in the serum preserved at −80°C altogether.

Markers of HBV infection

The HBeAg was estimated by enzyme-linked immunosorbent assay (ELISA) (F-HBe; Sysmex, Kobe). HBV DNA was determined by PCR followed by hybridization (Amplicor HBV Monitor: Roche Molecular Systems, Branchburg, NJ), and the results were expressed in log copy per milliliter over a range of 2.6–7.6. The 6 major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology) and the PCR-invader method with genotype-specific probes.[15] YMDD motif mutants were determined by PCR followed by restriction fragment length polymorphism (RFLP)[8] or enzyme-linked minisequence assay with commercial assay kits (PCR-ELMA; Genome Science).

Statistical analyses

Frequencies were compared between groups by the χ^2-test, Fisher's exact test, and HBV DNA values by Mann–Whitney *U*-test. Emergence of YMDD motif mutants and BTH were compared in the Kaplan–Meier life table by using the production limit method. A *P*-value < 0.05 was considered significant. Analyses of all data were performed with SAS 9.1.3.

RESULTS

DURING THE PERIOD of 12 years from 1995 to 2006, 929 HBV DNA-positive patients received 100 mg of lamivudine daily. From the total of 929 patients who received lamivudine for 1 year or more, 359 patients who maintained an ALT level of ≤ 40 IU/L were stratified based on the duration of lamivudine treatment and divided into 2 groups – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). Demographic features and clinical background of the two study groups were uniformly matched with no significant differences in age, sex, serum transaminase levels, HBV DNA, hepatitis B e-antigen (HBeAg), and HBV genotype (Table 2). The median ALT values were 112 IU/L and 145 IU/L in both the groups, respectively, and the median HBV DNA level was identical at 6.1 log copies/mL in both the groups.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for < 3 years

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine by ALT (IU/L) level was 27% in 53 patients maintaining an ALT level of ≤ 20 (group A), 58% in 46 patients maintaining an ALT level of ≤ 30 (group B); and 63% in 26 patients maintaining an ALT level of ≤ 40 (group C), with statistical differences among the 3 groups ($P = 0.002$). The incidence of BTH was 2% in group A, 7% in group B, and 48% in group C ($P < 0.001$). The lowest incidence of YMDD motif mutant and BTH was noted in patients with ALT level of ≤ 20 (IU/L) (Fig. 1a,b). Follow-up for patients who developed BTH was discontinued upon the detection of YMDD motif mutant.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine based on the HBV DNA (log copies/mL) level was 28% in patients maintaining an HBV DNA level of < 2.6; 83% in patients maintaining an HBV DNA level of 2.6–5.0; and 100% in patients maintaining an HBV DNA level of ≥ 5.1, with significant differences among the 3 groups ($P < 0.001$). The incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$) (Fig. 2a,b). The lowest incidence of YMDD motif mutant and BTH was seen in patients maintaining an HBV DNA level of < 2.6 log

Table 2 Background of 359 patients using lamivudine treatment for ≥ 1 year at the start of lamivudine therapy

Factors	Duration of lamivudine therapy		
	< 3 years $n = 125$	≥ 3 years $n = 234$	Differences (P-value)
Age (years)	23–75 (43)†	18–76 (43)†	NS‡
Male	93 (73%)	182 (77.1%)	NS‡
HBV infection in mother	47 (37%)	82 (35%)	NS‡
Chronic hepatitis	109 (85%)	212 (90%)	NS‡
AST (IU/L)	15–866 (80)†	19–2593 (83)†	NS‡
ALT (IU/L)	11–2092 (112)†	14–2142 (145)†	NS‡
Total bilirubin (mg/dL)	0.2–3.8 (0.7)†	0.2–10.6 (0.7)†	NS‡
γ-GTP (IU/L)	16–440 (54)†	13–468 (65)†	NS‡
HBV DNA (log copy/mL)	<2.6–>7.6 (6.1)†	<2.6–>7.6 (6.1)†	NS‡
HBeAg	66(52%)	107 (45%)	NS‡
HBV genotype (A, B, C, ND)	4:15:98:8	5:21:207:1	NS‡

†Median value where indicated. ‡Not significant. ALT, alanine transaminase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; γ-GTP, gamma glutamyl transferase.

copies/mL. The BTH incidence was particularly high in patients with an HBV DNA level of ≥ 5.1, which was 40% within 1 year.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine in patients based on both the ALT (IU/L) and HBV DNA (log copies/mL) level during the course of lamivudine treatment was evaluated (Table 3).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 7% and 2%, respectively. Whereas in patients with HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was higher at 16% and BTH was 0%, and in patients with ALT 31–40, YMDD motif mutant and BTH was further higher at 42% and 17%, respectively.

In patients with HBV DNA level at 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant was 33% in patients with 0% incidence of BTH. Nevertheless, in patients maintaining HBV DNA at 2.6–5.0 but with ALT 21–30, the incidence of YMDD motif mutant was 73% and BTH was 18%; whereas in patients with ALT 31–40, the incidence of YMDD motif mutant was 50% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, both YMDD motif mutant and BTH was 100%.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for ≥ 3 years

In patients treated with lamivudine for 3 years or more, the incidence of YMDD motif mutant by ALT (IU/L) level was 58% in 113 patients in group A, 60% in 84

Table 3 Incidences of tyrosine-methionine-aspartate-aspartate (YMDD) mutant and breakthrough hepatitis (BTH) by hepatits B virus (HBV) DNA and alanine transaminase (ALT) level in patients during lamivudine treatment for < 3 years (125 patients)

HBV DNA† (Amplicor: log copies/mL)	ALT level (IU/L)†					
	≤ 20		21–30		31–40	
	YMDD	BTH	YMDD	BTH	YMDD	BTH
< 2.6	3/41 (7%)	1/41 (2%)	5/32 (16%)	0/32 (0%)	5/12 (42%)	2/12 (17%)
2.6–5.0	4/12 (33%)	0/12 (0%)	8/11 (73%)	2/11 (18%)	6/12 (50%)	5/12 (42%)
≥ 5.1	0	0	3/3 (100%)	0/3 (0%)	2/2 (100%)	2/2 (100%)

†The HBV DNA and ALT levels are shown based on the treatment duration of lamivudine.

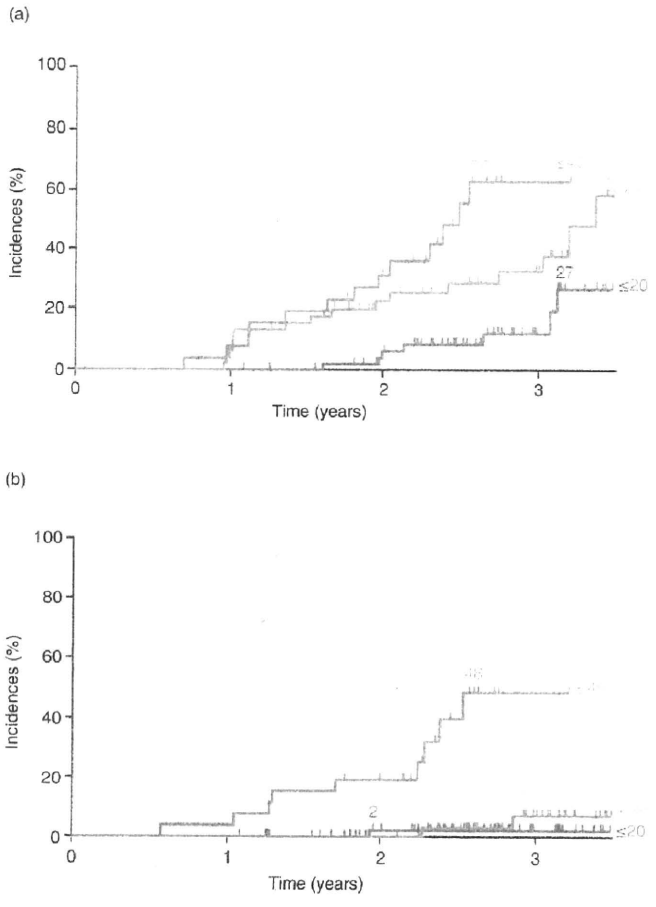

Figure 1 The incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatitis was noted in patients with alanine aminotransferase level of ≤ 20 (IU/L) (a) Incidence of YMDD mutants over time ($P = 0.0017$). (b) Incidence of break throughhepatitis over time ($P < 0.0001$).

patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$) (Fig. 3a,b).

In patients treated with lamivudine for ≥ 3 years, the increased incidence of YMDD motif mutant by HBV DNA (log copies/mL) level was 65% in patients maintaining an HBV DNA level of < 2.6, 78% in patients maintaining an HBV DNA level of 2.6–5.0, and 92% in patients maintaining an HBV DNA level of ≥ 5.1, and that of BTH in the corresponding groups was 10%, 18%, and 77% ($P < 0.001$) (Fig. 4a,b).

The incidence of YMDD motif mutant in ≥ 3 years treatment with lamivudine in patients by both ALT (IU/L) and HBV DNA (log copies/mL) levels during the course of lamivudine treatment was also analyzed (Table 4).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 38% and 7%, respectively. At the same HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was 48% and BTH was 8%; whereas at ALT 31–40, YMDD motif mutant was 36% and BTH was 9%.

In patients maintaining HBV DNA 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 60% and 4%, respectively. At the same HBV DNA

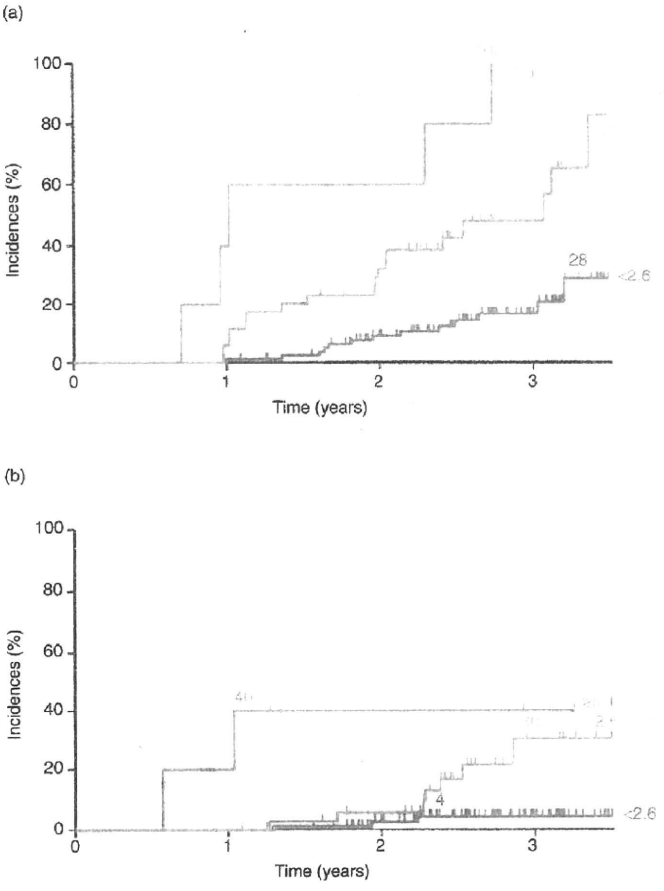

Figure 2 incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$). (a) Incidence of YMDD mutants over time ($P = 0.0001$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0037$).

level, 2.6–5.0 and ALT 21–30, the incidence of YMDD motif mutant was 86% and BTH was 18%; whereas at ALT 31–40, YMDD motif mutant was 92% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, YMDD motif mutant was 93% and BTH was 86%.

DISCUSSION

LONG-TERM THERAPY for CHBV can lead to the development of HBV drug-resistant mutants. Early detection of the YMDD motif mutants in lamivudine-treated patients and timely switch to other nucleoside analogues with low viral resistance is crucial to prevent viral and biochemical flares and ineffective therapeutic response. Although development of YMDD mutants results in decreased viral susceptibility to lamivudine, viral replication rate is lower in mutant strains than in wild type.[6]

Among the 359 patients who received lamivudine for > 1 year and maintained an ALT level of ≤ 40 IU/L, the rate of YMDD motif mutant was 11% (1 year), 29% (2 year), 42% (3 year), 49% (4 year) and 61% (5 year). BTH occurrences were 3% (1 year), 8% (2year), 13% (3 year), 15% (4 year) and 19% (5 year). The rate of

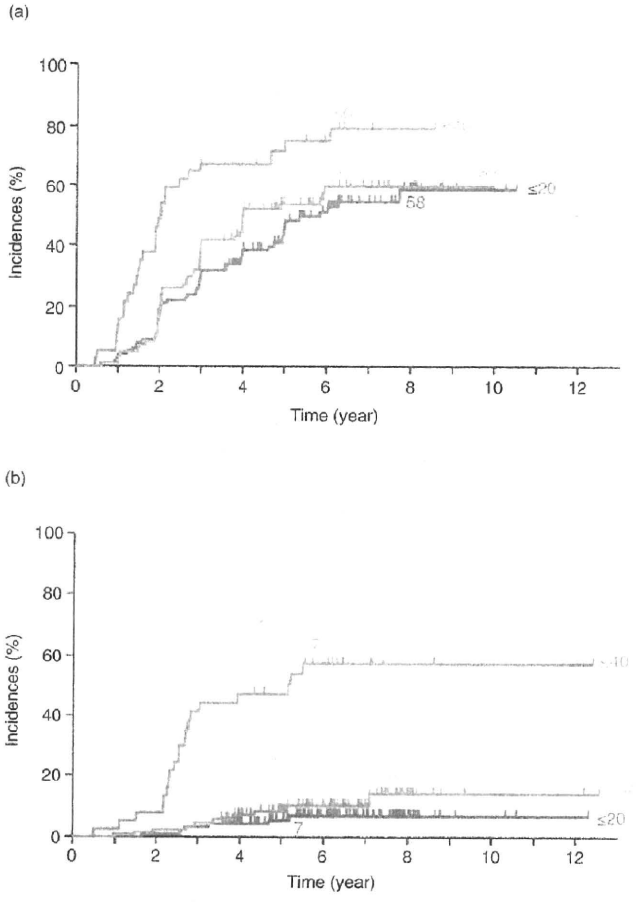

Figure 3 In patients treated with lamivudine for 3 years or more, the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant by alanine aminotransferase (IU/L) level was 58% in 113 patients in group A, 60% in 84 patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$). (a) Incidence of YMDD mutants over time ($P = 0.0015$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0001$).

YMDD motif mutant and BTH were low after 3 or more years of treatment with lamivudine. Therefore, the year of switching treatment from lamivudine to other nucleic acid analogue will be at 3 years. Accordingly, in this study, we examined patients treated with lamivudine for < 3 and ≥ 3 years.

Among the patients treated with lamivudine for < 3 years, the lowest incidence of YMDD motif mutant and BTH was seen in patients with ALT < 20 IU/L maintaining HBV DNA level of 2.6–5.0. The other category for lowest incidence was in patients with ALT 21–30 IU/L and HBV DNA level of < 2.6 log copies/mL. In this study, within 3 years of treatment with lamivudine, the group of patients with the recommended HBV DNA (< 2.6 log copies/mL) and ALT maintained at 21–30 IU/L may be considered eligible to be switched to entecavir therapy as per Japanese guidelines. We, however, believe it is important to consider the prognosis for patients who are switched from lamivudine to entecavir. Similarly, in patients maintaining HBV DNA level in the range of 2.6–5.0 log copies/mL and ALT < 20 IU/L, switching to dual therapy with adefovir in combination with lamivudine depends on the related viral breakthrough. In a study by Li Zhou *et al.*,[16] some patients with YMDD motif mutants had significantly lower HBV DNA and ALT levels compared with baseline

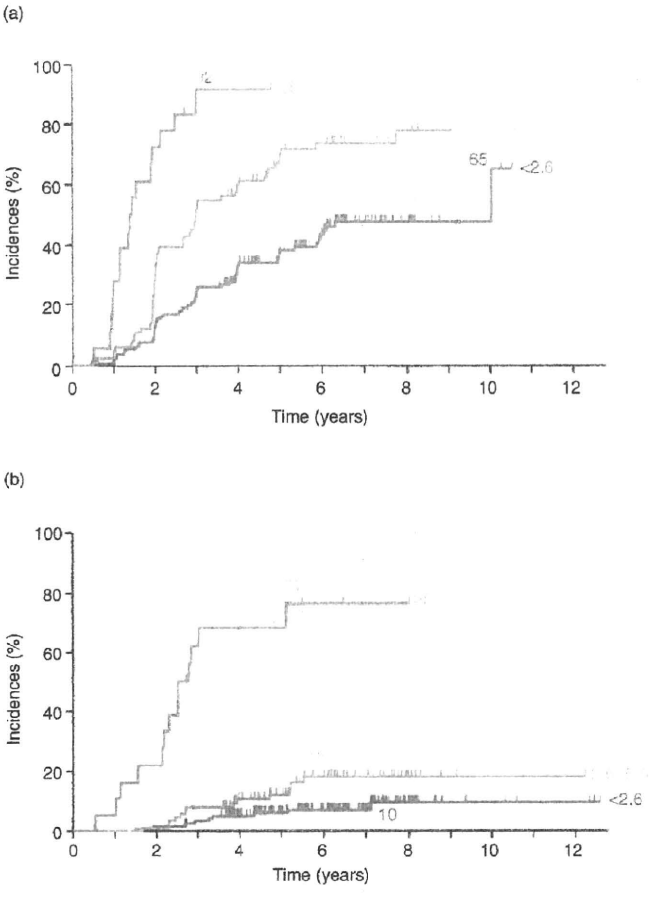

Figure 4 In patients treated with lamivudine for ≥ 3 years, the increased incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant by hepatitis B virus (HBV) DNA (log copies/mL) level was 65% in patients maintaining an HBV DNA level of < 2.6, 78% in patients maintaining an HBV DNA level of 2.6–5.0, and 92% in patients maintaining an HBV DNA level of ≥ 5.1, and that of BTH in the corresponding groups was 10%, 18%, and 77% ($P < 0.001$). (a) Incidence of YMDD mutants over time ($P = 0.0001$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0001$).

values, which might be due to decreased replication efficiency of the HBV mutants.

HBeAg, severe liver disease, high HBV DNA, and low ALT levels at the baseline were factors accelerating the development of BTH. This was in confirmation of previous results.[17–19] Development of BTH, however, was not influenced by HBV genotypes. This is probably due to the response in HBeAg-positive patients, which was comparable among those with different genotypes though it differed among HBeAg-negative patients.[20]

In a study of Japanese adult patients treated with lamivudine for > 12 months, the YMDD motif mutation was detected in 26% patients, with 23, 16, and 21 patients correspondingly positive for YIDD, YVDD, and YIDD + YVDD mutants. The occurrence of mutations steadily increased and two, five, and 52 patients with genotypes A, B, and C, respectively developed resistance.[21] Lamivudine retreatment could induce rapid re-emergence of YMDD motif mutants with associated viral and hepatic flares[22] and should be avoided. Next, we were interested to know if any difference in sensitivity existed in detecting YMDD mutants by the two different methods used in this study, PCR-RFLP and PCR-ELMA. We studied the rate of detection of YMDD motif mutant by both methods in 20 patients who received lamivudine for more than two years. The detection rate

Table 4 Incidences of tyrosine-methionine-aspartate-aspartate (YMDD) mutant and breakthrough hepatitis (BTH) by hepatitis B virus (HBV) DNA and alanine transaminase (ALT) level in patients during lamivudine treatment for ≥ 3 years (234 patients)

HBV DNA† (Amplicor: log copies/mL)	ALT level (IU/L)†					
	≤ 20		21–30		31–40	
	YMDD	BTH	YMDD	BTH	YMDD	BTH
< 2.6	23/60 (38%)	4/60 (7%)	29/61 (48%)	5/61 (8%)	4/11 (36%)	1/11 (9%)
2.6–5.0	30/50 (60%)	2/50 (4%)	19/22 (86%)	4/22 (18%)	11/12 (92%)	5/12 (42%)
≥ 5.1	3/3 (100%)	1/3 (33%)	0/1 (0%)	0/1 (0%)	13/14 (93%)	12/14 (86%)

†The HBV DNA and ALT levels are shown based on the treatment duration of lamivudine.

between PCR-RFLP and PCR-ELMA was similar; eight patients (40%) and nine patients (45%), respectively.[23]

CONCLUSION

CORRELATION OF ALT and HBV DNA levels with YMDD motif mutant and viral breakthrough can be used as an indirect method of estimating susceptibility to develop lamivudine resistance. The low incidence of YMDD motif mutant and BTH associated with an HBV DNA level of < 2.6 log copies/mL and ALT level of ≤ 30 IU/L and an HBV DNA level of 2.6–5.0 log copies/mL and ALT level of ≤ 20 IU/L during only less than 3 year-treatments can be utilized as a clinically relevant tool to monitor patients' criteria in switching to other nucleoside analogue drugs. Using these simple methods, which can be easily pursued in clinical practice, it may be feasible in the future to switch from lamivudine to other nucleoside analogue drugs with low rates of inducing resistant mutants in CHBV patients. This is important considering the risk of continuous lamivudine treatment causing YMDD motif mutant and BTH.

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Original Article

Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants

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Aim: To identify factors for the development of hepatocellular carcinoma (HCC) in the patients who receive adefovir add-on lamivudine for treatment of lamivudine-resistant hepatitis B virus (HBV) mutants.

Methods: A total of 247 patients who developed lamivudine-resistant HBV mutants, with an increase of HBV DNA ≥ 1 log copies/mL, received adefovir dipivoxil 10 mg add-on lamivudine 100 mg daily during a median of 115 weeks (range: 25–282 weeks). They were followed for the development of HCC by imaging modalities every 3–6 months.

Results: HCC developed in 18 of the 247 (7.3%) patients. Eight factors were in significant association with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase and α-fetoprotein, as well as YMDD mutants at the start of adefovir dipivoxil. By the multivariate analysis, AST levels, YIDD mutants, cirrhosis and age were independent factors for the development of HCC. By the Kaplan-Meier analysis, AST levels ≥ 70 IU/L, YIDD mutants, cirrhosis and age ≥ 50 years increased the risk of HCC ($P = 0.018$, $P = 0.035$, $P = 0.002$ and $P = 0.014$, respectively). HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$).

Conclusion: HCC can develop in cirrhotic patients receiving adefovir add-on lamivudine. Hence, the patients with baseline AST ≥ 70 IU/L and YIDD mutants would need to be monitored closely for HCC.

Key words: adefovir dipivoxil, chronic hepatitis B, hepatitis B virus, hepatocellular carcinoma, lamivudine, rescue therapy

INTRODUCTION

WORLDWIDE, AN ESTIMATED 400 million people are infected with hepatitis B virus (HBV) persistently, and one million die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually.[1,2] Interferon (IFN) was introduced for treatment of chronic hepatitis B, and it has been replaced for pegylated-IFN.[3] Due to substantial side-effects and requirement for injection, however, IFN-based therapies are not favored.

In 1998, lamivudine was approved as the first nucleot(s)ide analogue for treatment of chronic hepatitis B,[4] and then adefovir in 2002.[5] Due to its lower costs and safety records, lamivdine has gained a wide popularity for treatment of chronic hepatitis B. However, drug-resistant mutants arise in parallel with the duration of lamivudine, in 12.5% after 1 year, in 43.8% after 3 years, and 62.5–70.2% after 5 years.[6,7] For preventing breakthrough hepatitis induced by lamivudine-resistant HBV mutants, additional adefovir dipivoxil 10 mg daily has been recommended;[8,9] it is more effective than switching to adefovir monotherapy and has fewer chances of developing drug-resistant mutants.[10,11]

Since 1995, 930 patients with chronic hepatitis have been treated with lamivudine in the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo.[12] HBV mutants with mutations in the thyrosine-methionine-aspartic acid-aspartic acid (YMDD) motif elicited in the 247 (26.5%) patients, and they started to receive additional adefovir since December, 2002.[13,14] However, HCC developed in 18 (7.3%) of them during the combination therapy for 25–282 weeks; HCC has

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not been reported in any of the patients who have received adefovir add-on lamivudine for 5 years.[15–17] Hence, factors for the development of HCC in the patients receiving adefovir add-on lamivudine were sought for in a retrospective study.

METHODS

Patients

OVER A PERIOD of 13 years, from September 1995 to September 2007, 930 patients with chronic hepatitis B received long-term lamivudine treatment at the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Drug-resistant YMDD mutants developed in 247 (26.5%) of them, accompanied by an increase in HBV DNA ≥ 1 log copies/mL, and they received adefovir 10 mg in addition to lamivudine 100 mg daily during the median of 115 weeks (range: 25–282 weeks). They have been followed for liver function and virological markers of HBV infection monthly, as well as blood counts and tumor makers including alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II). Cirrhosis was diagnosed by laparoscopy or liver biopsy, and in the patients who had not received them, by clinical data, imaging modalities and portal hypertension. HCC was diagnosed by hypervascularity on angiography and/or histological examination, characteristic features of computed tomography, magnetic resonance imaging and ultrasonography. An informed consent was obtained from each patient in this study, and the protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the institution's human research committee.

Markers of HBV infection

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay (ELISA) with commercial kits (HBeAg EIA, Institute of Immunology, Tokyo). HBV DNA was quantitated by the Amplicor monitor assay (Roche Diagnostics, Tokyo) with a dynamic range over 2.6–7.6 log copies/mL. Genotypes of HBV were determined serologically by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the seven major genotypes (A–G),[18,19] with use of commercial kits (HBV Genotype EIA, Institute of Immunology).

Detection of YMDD mutants

YMDD mutants were determined by polymerase chain reaction (PCR)-based enzyme-linked mini-sequence assay (PCR-ELIMA) with commercial kits (Genome Science Laboratories, Tokyo).

Statistical analyses

Categorial variables were compared between groups by the χ^2 test, and non-categorical variables by the Mann–Whitney *U*-test. A *P*-value < 0.05 was considered significant. Factors associated with HCC by univariate analysis were evaluated by the multivariate analysis by the stepwise Cox proportional hazard model. Development of HCC with time was analyzed by the Kaplan–Meier method, and differences were evaluated by the log-rank test. Data were analyzed by the SPSS software, version 11.0 (Chicago, IL).

RESULTS

Baseline characteristics of the patients who did and who did not develop hepatocellular carcinoma during adefovir add-on lamivudine treatment

TABLE 1 COMPARES characteristics at the start of adefovir between the 18 patients who developed HCC and the 229 who did not. Eight factors were associated with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, bilirubin, AST, alanine aminotransferease (ALT) and α-fetoprotein (AFP) levels, as well as YMDD mutants. HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$). There were 61 (26.6%) patients who had cirrhosis at the start of adefovir. Of them, one of the 18 (2.2%) with HCC and 18 of the 229 (2.2%) without HCC presented with decompensation; no patients developed decompensation after the start of adefovir.

Rates of HBV DNA disappearance from serum (< 2.6 log copies/mL) were: 55% (113/207) at 1 year, 71% (119/168) at 2 years, 77% (78/101) at 3 years and 85% (35/41) at 4 years. Rates of AST normalization (< 38 IU/L) were: 87% (179/207) at 1 year, 90% (151/168) at 2 years, 92% (93/101) at 3 years and 95% (39/41) at 4 years; and those of ALT normalization (< 50 IU/L) were: 88% (183/207) at 1 year, 91% (153/168) at 2 years, 93% (94/101) at 3 years and 98% (40/41) at 4 years. There were no differences in the rate of HBV DNA disappearance from serum between the patients with and without HCC: 57% (8/14) vs. 54% (105/193) at 1 year ($P = 1.0$); 86% (12/14) vs. 70% (107/154) at 2 years ($P = 0.229$); and 89% (8/9) vs.

Table 1 Characteristics of patients who did and did not develop hepatocellular carcinoma (HCC) at the start of adefovir†

	HCC developed ($n = 18$)	HCC did not develop ($n = 229$)	Differences *P*-value
Duration of lamivudine before the start of adefovir	128 (31–346)	144 (13–617)	0.321
Age (years)	52 (35–75)	45 (26–75)	0.008
Men	15 (83%)	183 (80%)	1.000
Cirrhosis	10 (56%)	51 (22%)	0.004
Platelets ($\times 10^3/mm^3$)	12.0 (4.6–19.7)	16.3 (3.1–31.9)	0.001
Albumin (g/dL)	3.6 (2.3–4.7)	3.9 (2.8–4.7)	0.073
Bilirubin (mg/dL)	0.8 (0.5–15.5)	0.7 (0.2–6.0)	0.046
Creatinine (mg/dL)	0.8 (0.5–1.0)	0.8 (0.4–1.6)	0.950
AST (IU/L)	119 (55–248)	66 (14–1413)	0.003
ALT (IU/L)	151 (61–576)	104 (13–1563)	0.035
AFP (ng/dL)	8 (2–130)	4 (1–282)	0.026
HBV genotypes			0.228
C	18 (100%)	189 (87%)	
Others	0	27 (13%)	
HBeAg	8 (44%)	132 (58%)	0.323
HBV DNA (log copies/mL)	7.1 (4.4–>7.6)	7.1 (<2.6–>7.6)	0.623
YMDD mutants			0.041
YIDD	13 (72%)	109 (45%)	
YVDD	5 (28%)	62 (25%)	
YI/VDD	0	56 (23%)	

†Values are the median with the range in parentheses or *n* with percent in parentheses.
AFP, alpha-fetoprotein; ALT, alaine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

92% (85/92) at 3 years ($P = 0.555$). Rates of normalized AST levels in the patients with and without HCC were: 50% (7/14) vs. 90% (173/193) at 1 year ($P < 0.001$); 79% (11/14) vs. 91% (140/154) at 2 year ($P = 0.166$); and 67% (6/9) vs. 95% (87/92) at 3 year ($P = 0.037$). Rates of ALT normalization in the patients with and without HCC were: 71% (10/14) vs. 90% (174/193) at 1 year ($P = 0.037$); 79% (11/14) vs. 90% (139/154) at 2 year ($P = 0.189$); and 56% (5/9) vs. 92% (85/92) at 3 year ($P = 0.015$). Thus, normalization of AST and ALT was less frequent in the patients with than without HCC.

Characteristics of the 18 patients who developed HCC are compared between the baseline and at the development of HCC (Table 2). At the start of adefovir, 10 (56%) of them had developed cirrhosis and 16 (89%) had AST levels ≥ 70 IU/L. HBV DNA was not detectable in 10 (56%) of them at the development of HCC. Of the eight patients with detectable HBV DNA levels (≥ 2.6 log copies/mL), five (63%) developed HCC within 1 year after the start of adefovir. AST was elevated (> 38 IU/L) in eight patients, including four (50%) without detectable HBV DNA levels.

Factors independently associated with the development of hepatocellular carcinoma

Eight factors associated with the development of HCC by the univariate analysis, including age, cirrhosis, platelet counts, bilirubin, AST, ALT and AFP levels, as well as YMDD mutants (Table 1), were evaluated by the multivariate analysis. AST ≥ 70 IU/L, YIDD mutants, age ≥ 50 years and cirrhosis at the baseline were independent risk factors for the development of HCC (Table 3). There were no differences in the distribution of YIDD, YVDD and the mixture thereof among the patients with distinct AST, ALT or HBV DNA levels or between those with and without cirrhosis at the start of adefovir. HBV mutants with mutations resistant to adefovir (rtA181T/S, rtN236T) occurred in two of the 247 (0.8%) patients; none of them developed HCC.

The median time between the elevation of HBV DNA > 5.0 log copies/mL and the administration of adefovir was 124 (range: 0–815) days for the 13 patients who developed HCC and 147 (0–3268) days for the 166 patients who did not ($P = 0.605$). The median time between the elevation of ALT > 43 IU/L and the start of

Table 2 Characteristics of the 18 patients at commencement of adefovir (ADV) and development of hepatocellular carcinoma (HCC)

Patient no.	Age (years)	Sex	At the commencement of ADV					YMDD mutant	Period of ADV (years)	At the development of HCC		
			Liver disease	AST (IU/L)	ALT (IU/L)	HBeAg	HBV DNA (log copies/mL)			AST (IU/L)	ALT (IU/L)	HBV DNA (log copies/mL)
1	50	M	CH	248	576	–	6.9	I	4.5	26	27	< 2.6
2	35	M	LC	217	164	+	7.5	I	1.6	54	34	< 2.6
3	50	M	LC	192	272	+	> 7.6	I	1.2	68	89	< 2.6
4	61	M	CH	192	332	–	6.9	I	2.8	22	23	< 2.6
5	65	M	CH	174	219	–	5.2	V	0.1	30	43	< 2.6
6	58	M	CH	160	216	–	6.5	V	2.2	41	32	< 2.6
7	53	M	LC	127	97	+	> 7.6	I	0.5	55	41	3.2
8	75	M	LC	119	209	+	> 7.6	V	1.1	121	125	2.6
9	58	F	CH	118	214	+	4.4	I	3.3	21	13	< 2.6
10	48	M	CH	116	99	+	> 7.6	I	3.3	32	36	< 2.6
11	51	F	LC	111	130	–	5.3	I	0.9	88	95	< 2.6
12	47	M	CH	85	138	+	> 7.6	I	1.3	28	29	3.1
13	61	M	LC	81	65	–	5.6	I	0.2	32	27	2.9
14	59	F	LC	80	132	–	> 7.6	V	0.1	32	41	3.2
15	40	M	LC	75	124	–	6.3	I	3.8	21	24	< 2.6
16	48	M	CH	71	61	–	6.6	I	0.6	48	26	3.7
17	55	M	LC	55	76	+	7.3	I	0.2	50	64	5.4
18	43	M	LC	27	21	–	5.4	V	1.6	30	23	3.7

ALT, alaine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; I, YIDD mutant; LC, cirrhosis; V, YVDD mutant.

Table 3 Independent risk factors influencing the development of hepatocellular carcinoma

Factors	Category	Hazard ratio (95% CI†)	*P*-value
AST (IU/l)	1: < 70	1	0.016
	2: ≥ 70	6.21 (1.40–27.5)	
YMDD mutants	1: YVDD or YV/IDD	1	0.012
	2: YIDD	3.97 (1.36–11.6)	
Age (years)	1: < 50	1	0.023
	2: ≥ 50	3.24 (1.17–8.95)	
Cirrhosis	1: Absent	1	0.030
	2: Present	1.42 (1.04–1.96)	

†Confidence interval.

adefovir was 59 (0–896) days for the patients who developed HCC and 54 (0–3240) days for those who did not ($P = 0.330$). Hence, exacerbation of hepatitis was not a risk factor for the development of HCC.

Age-specific risk factors for the development of HCC were evaluated by the multivariate analysis. In the patients < 50 years, platelet counts < $13 \times 10^3/mm^3$ was the only significant risk factor for HCC (hazard ratio 6.88 [95% confidence interval; 1.26–37.6]), while AST levels ≥ 70 IU/L was that in those ≥ 50 years (hazard ratio: 9.50 [95% confidence interval 1.20–74.9]).

Factors increasing the cumulative incidence of hepatocellular carcinoma

AST levels ≥ 70 IU/L at the start of adefovir increased the development of HCC during follow-ups ranging to 5 years (Fig. 1). HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD mutants (Fig. 2). The cumulative incidence of HCC in the patients with YIDD mutants alone was: 4% at 1 year, 10% at 3 years and 43% at 5 years. In contrast, HCC never developed in the patients with the mixture of YIDD and YVDD mutants through 5 years of follow-up. HCC developed more frequently in the patients with cirrhosis and those aged ≥ 50 years (Figs 3,4, respectively).

DISCUSSION

HCC DEVELOPED IN 18 of the 247 (7.3%) patients who had received adefovir add-on lamivudine during a long-term ranging to 5 years. There were some differences in the characteristics at the start of adefovir dipivoxil between the patients who did and who did not develop HCC. The patients who developed HCC were older, more frequently had signs of early cirrhosis with less platelet counts, as well as higher levels of AST, ALT and AFP, than those who did not develop HCC. By multivariate analysis, AST ≥ 70 IU/L, YIDD mutants in

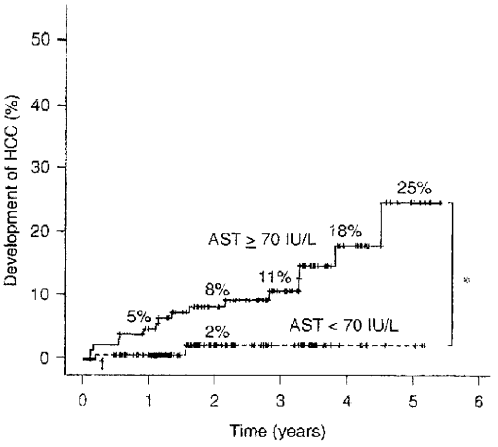

Figure 1 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with different baseline aspartate aminotransferase (AST) levels. *$P = 0.009$.

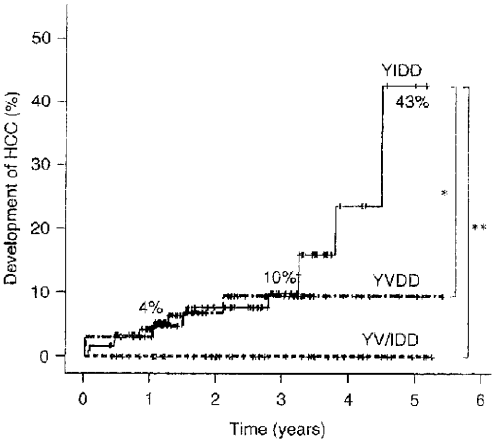

Figure 2 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with distinct YMDD mutants. *$P = 0.035$; **$P = 0.003$.

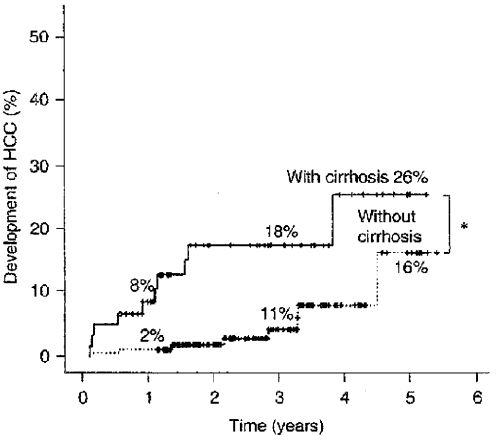

Figure 3 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with and without cirrhosis at the baseline. $^*P = 0.002$.

comparison with YVDD or the mixture of YVDD and YIDD mutants, age ≥ 50 years and cirrhosis were independent risk factors for the development of HCC. By the Kaplan-Meier life-table analysis, the cumulative incidence of HCC during 5 years in the patients receiving

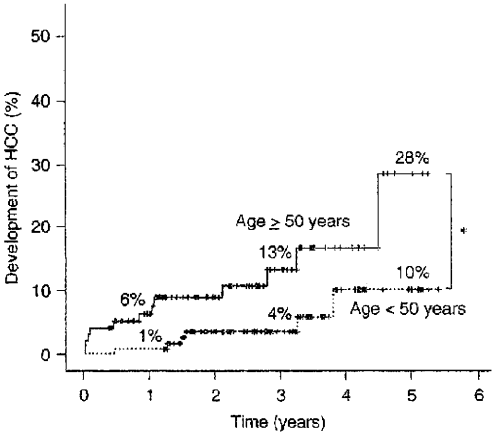

Figure 4 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients aged ≥ 50 years and < 50 yeas at the baseline. $^*P = 0.014$.

adefovir add-on lamivudine was significantly higher in those with AST ≥ 70 IU/L, YIDD mutants, cirrhosis and aged ≥ 50 years at the start of adefovir.

A marked difference in the development of HCC between the present study (7.3% [18/247]) and two studies reported from Europe and the US (0/70 and 0/65, respectively)[16,17] would be accounted for, at least in part, by the age of patients who developed HCC in this study that was older than in those in previous reports (the median of 52 years vs. means of 36 and 47 years, respectively). This view would be supported by the age of patients with long-term adefovir add-on lamivudine that was higher in those with than without the development of HCC (52 vs. 45 years [median], $P = 0.008$). HBV infection in Asia is acquired by the perinatal infection, while that in Western countries is gained after the adolescence ~20 years after birth. Hence, the duration of HBV infection would have been > 20 years longer in Japanese than Western patients. In addition, genotypes of HBV may give an additional account on the difference in development of HCC between them. All the 18 patients who developed HCC in this study were infected with genotype C; it is associated with HCC more closely than the other genotypes.[20–23] By contrast, by far the most patients from Western countries would have been infected with genotypes A and D.[24,25]

HCC developed more frequently in patients with than without cirrhosis at the start of adefovir (10/61 [16.4%] vs. 8/186 [4.3%], $P = 0.002$). Hence, cirrhosis increased the risk of HCC in patients receiving adefovir add-on lamivudine. This view is supported by the development of HCC in 11 of the 94 (11.7%) patients with cirrhosis who received adefovir add-on lamivudine from Italy.[10] Although HCC did not develop in any of the 39 Italian patients with chronic hepatitis, it did in eight of the 186 (4.3%) Japanese patients in the present study. There were, however, marked differences in the median baseline ALT levels between Italian and Japanese patients (58 vs. 108 IU/L); the grade of liver inflammation would have been higher in the Japanese patients. In actuality, all the eight patients with chronic hepatitis who developed HCC had high AST and ALT levels at the start of adefovir (Table 2).

In the natural history of persistent HBV infection, HCC develops more frequently in the patients with persistently high ALT levels than in those with normal levels. Hence, necroinflammation in the liver would contribute to carcinogenesis.[26,27] Although adefovir add-on lamivudine may prevent virological breakthroughs, it would not be able to suppress the pre-

neoplastic state induced by exacerbation of hepatitis. It would be necessary therefore to identify the patients with chronic hepatitis at an increased risk for HCC during adefovir add-on lamivudine, such as those with cirrhosis or aged ≥ 50 years, and take special care of them toward early detection of HCC and immediate therapeutic intervention. They need to be monitored frequently for any increase in HBV DNA and aminotransferase levels that herald breakthrough hepatitis during lamivudine therapy.

In the present study, HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD; there have been no studies correlating YMDD mutants and the development of HCC. No patients with the mixture of YVDD and YIDD mutants developed HCC, despite the predominance of YIDD mutants in the patients with HCC. This might have been due to the assay used for YMDD mutants by the commercial kit; it can miss YVDD mutants in samples in which YIDD mutants account for the great majority. By the assay method specific for either mutant, YIDD was detected either alone or accompanied by small amount of YVDD in the patients who have received adefovir add-on lamivudine treatment.[28] Sensitive and specific quantification of YIDD and YVDD mutants are necessary for further evaluating a role for YIDD mutants in hepatocarcinogenesis, as well as for identifying factors promoting the generation of both YIDD mutants and HCC.

Some points of clinical importance have emerged in the present study. First, patients who receive a long-term adefovir add-on lamivudine and have developed YMDD mutants need to be screened for HCC on the regular basis. This is required especially for the patients who have signs of cirrhosis and/or high AST levels, or aged ≥ 50 years. In these high-risk patients, adefovir has to be started promptly when HBV DNA levels increase, even before transaminase levels elevate in them. Secondly, it would be a matter of concern if adefovir is involved in the development of HCC. Should it be the case, tenofovir or newer potent antivirals, either as a monotherapy or add-on lamivudine, would deserve considerations. Thirdly, it needs to be evaluated if YIDD mutants have any significance in the development of HCC. Although nucleot(s)ide analogues may suppress hepatic inflammation and are expected to improve the prognosis of patients with chronic hepatitis B, they need to be monitored closely for HCC. The development of HCC has to be identified, as early as possible, for timely treatment toward longevity with minimal morbidity and improvement of the quality of life.

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Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Affect Very Early Viral Dynamics During Treatment With Telaprevir, Peginterferon, and Ribavirin

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Substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the rate of HCV RNA loss following 12-week triple therapy, and determine the effect of aa substitutions on very early (within 48 hr) viral dynamics. Sixty-seven patients infected with HCV genotype 1b (HCV-1b) and high viral load who received 12-week triple therapy were studied. RNA loss could be achieved in 2%, 34%, 80%, 92%, 95%, 94%, and 90% of the patients after 1, 2, 4, 6, 8, 10, and 12 weeks of triple therapy, respectively. After 24-hr treatment, the proportion of patients with Arg70 and Leu91 substitutions with ≥3.0 log fall in HCV RNA was significantly higher than those with <3.0 log fall ($P = 0.008$). However, the aa substitution patterns in the core region did not influence the fall in HCV RNA after 48-hr treatment. Multivariate analysis identified substitutions of aa 70 and 91 ($P = 0.014$) and level of viremia at baseline (≥7.0 log IU/ml; $P = 0.085$) as independent parameters that determined the ≥3.0 log fall in HCV RNA level after 24-hr triple therapy. It is concluded that 12-week triple therapy achieved high rates of loss of HCV RNA in Japanese patients infected with HCV-1b and high viral load, and that the aa substitution pattern in the core region seems to influence very early viral dynamics. ***J. Med. Virol. 82:575–582, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: HCV; core region; NS5A-ISDR; telaprevir; peginterferon; ribavirin; very early viral dynamics

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999]. At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) with high viral loads (>100 KIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Iino et al., 2005; Tsubota et al., 2005]. Such background calls for efficient treatment of patients with chronic HCV infection.

Even with pegylated interferon (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b with high viral loads [Manns et al., 2001; Fried et al., 2002]. Recently, a new strategy was introduced for the treatment of chronic HCV infection by inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these drugs, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [Lin et al., 2006]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, results in a robust antiviral activity [Modi and Hoofnagle, 2007; Zeuzem, 2008]. Specifically, HCV RNA disappears in

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almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin [Lawitz et al., 2008; Suzuki et al., 2009]. However, patients resistant to treatment who do not achieve sustained virological response by the triple therapy, have been reported [Lawitz et al., 2008; Hézode et al., 2009; McHutchison et al., 2009]. The underlying mechanism of the response to the treatment is still not clear.

It is useful to evaluate treatment efficacy based on viral dynamics as an early predictor of PEG-IFN plus ribavirin combination therapy. Previous reports showed that decreases in HCV RNA levels were significantly greater in patients with than without sustained virological response from 24 hr to 12 weeks after the start of PEG-IFN plus ribavirin combination therapy in patients infected with HCV-1b and high viral load. Very early dynamics within 48 hr of such treatment is particularly important for early prediction of response to therapy [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Accordingly, the pretreatment predictors of very early dynamics during triple therapy of telaprevir with PEG-IFN and ribavirin were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with genotype 1b and high viral load are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009], and also affect the clinical outcome, including insulin resistance and hepatocarcinogenesis [Akuta et al., 2007c, 2009b; Fishman et al., 2009; Nakamoto et al., 2009]. However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict the very early dynamics and response to triple therapy of telaprevir with PEG-IFN and ribavirin.

The present study included 67 patients with HCV-1b and high viral load, who received triple therapy of telaprevir with PEG-IFN plus ribavirin and followed-up for 12 weeks or more after the start of treatment. The aims of the study were to determine the rate of loss of HCV RNA during treatment, and to identify the pretreatment factors that could predict very early viral dynamics (within 48 hr) after the start of treatment, including aa substitutions in the HCV core, the NS3, and the NS5A regions.

PATIENTS AND METHODS

Study Patients

Between May 2008 and May 2009, 67 patients infected with HCV were recruited to the study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (30%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN, and ribavirin], and 47 patients (70%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks. All patients were followed-up for at least 12 weeks after the start of triple therapy.

All patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C; (2) HCV-1b confirmed by sequence analysis; (3) HCV RNA levels of $\geq$5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese (Mongoloid) ethnicity; (5) age at study entry of 20–65 years; (6) body weight $\geq$35 and $\leq$120 kg at the time of registration; (7) lack of decompensated cirrhosis; (8) absence of hepatitis B surface antigen (HBsAg) in serum; (9) no history of HCC; (10) no previous treatment for malignancy; (11) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C; (12) no history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of $\leq$50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of $\geq$110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline; and (13) hemoglobin level of $\geq$12 g/dl, neutrophil count $\geq$1,500/mm^3, and platelet count of $\geq$100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study.

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at a dose of 750 or 500 mg three times a day at an 8-hr (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, NJ) was injected subcutaneously with a median dose 1.5 μg/kg (range: 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1,000 mg). All participating patients received these three drugs in the initial 12 weeks of the study.

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutrophil count below 750/mm^3, or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3, or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800–600, and 1,000–600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir